IN THE UNITED STATES PATENT OFFICE

In the application of
ROCHUS JONAS ET AL.
Serial No.: 043,925
Filed: May 30, 1979
For: PHENOXY-AMINO-PROPANOLS

Group: 125
Examiner: Robinson

D E C L A R A T I O N

KARL-HEINZ BECKER, being duly warned, deposes and says:

THAT he is a citizen of Germany, residing at Dieburg, Germany;

THAT he is a veterinarian by training and experience;

THAT he received the degree of Dr. med. vet. (doctor of veterinary medicine) from the University of Gießen in 1964;

THAT he has been working as pharmacologist in the Medical Research department of E. Merck, Darmstadt, Germany, since 1970;

THAT he is the author or co-author of numerous papers and patents in the fields of cardiovascular pharmacology;

THAT he has carried out, or supervised, respectively, experiments to compare the protection against myocardial injury, ensuing from coronary artery occlusion of the following:

I 1-(p-2-isopropoxyethoxymethyl-phenoxy)-3-isopropylamino-propan-2-ol

II 1-(p-2-isopropoxyethoxy-phenoxy)-3-isopropylamino-propan-2-ol

THAT compound I is an object of the present application disclosed in Example 46 and claimed in Claim 6 (fourth compound); compound II is a substance known from U.S. patent no. 3,674,840, example 8;

THAT, in detail the protection against myocardial injury was determined by the following test method which is similar to that described in detail in the paper of MAROKO et al., Circulation, vol. XLIII (1971), pages 67 to 82;

Principle

Partial hypoxy of the myocardium may be induced in dogs by reversible occlusion of the coronary artery. The increase in the ST segment in the electrograms traced from this hypoxic area indicates the extent of the lesion in the myocardial tissue. Various therapeutic agents, e.g. beta-blockers, are capable of reducing these sequels of hypoxy.

The experiments are to test whether the trial substance is able to exert a favorable influence on the pathological changes in the ST segment induced by coronary occlusion.

Preparation of the animals

As test animals, there are used male or female dogs of different age (15 to 25 kg weight).

These are deprived of food 24 hours before starting the experiment; they receive, however, water ad libitum. The dogs are anesthetized with 100 mg/kg of chloralose intravenously and fixed on an operation table.

A metal canula is fixed into the trachea and polyethylene catheters of 1.0 to 1.5 mm interior diameter are fixed into the A. and V. femoralis and via the right A. brachialis into the aorta ascendens.

Thereafter, the dogs are relaxed with 40 mg (per animal) of gallamine intravenously. They are artifically respirated by means of a respiration pump with room air at a frequency of 15 per minute so that the final expiratory carbon dioxide concentration is between 4.5 and 5 per cent (per volume).

Subsequently the R. interventricularis is exposed about 1 to 2 cm behind the division of the left coronary artery in order to attach an electromagnetic blood flow recorder and a pneumatic occlusion cuff (own construction) distal to the recorder. The A. carotis and V. jugularis are also exposed in order to introduce a catheter or an atrial electrode (own construction).

In the supply area of the exposed coronary artery, a ring electrode (platinum wire 0.3 mm thick, internal ring diameter 2 mm, (own construction) is fastened centrally with

two ligatures at the epicardium using atraumatic material (2/0 surgical silk, needle RB-1, round body, Ethicon, Norderstedt).

Treatment

A single dose of the trial substance is injected into the V. femoralis of an experimental animal during 2 min. The injection volume is 0.1 ml/kg; the solvent used is isotonic NaCl solution. After each injection the animal receives an additional amount of 0.5 ml/animal of isotonic NaCl solution. As a function test, adenosine, dissolved in isotonic NaCl solution, is asministered intravenously in a volume of 0.1 ml/kg in 10 sec.

In order to induce regional ischaemia in the myocardium, the coronary artery is occluded for 60 to 120 sec. each time.

Recording

The pathological changes in the epicardial electrogram (epicardial electrode against right foreleg) as well as the electrocardiogram lead in the right atrium (the electrode is pushed into rhe right atrium via the V. jugularis under radio-optical control) against the left hindleg are measured using electrocardiogram preamplifiers EC-592 (Schwarzer GmbH, Munich).

Evaluation

In the evaluation, the values obtained after administration of the trial substance are compared with the initial values and the absolute and percent (proportional) changes as well as the duration of action are recorded.

THAT the following results have been obtained:

With 0.125 mg/kg of the present substance I, an initial ST segment elevation of 10.0 mV caused by occlusion alone, was decreased to

2.0 mV after 10 minutes (80 % decrease);

1.5 mV after 20 minutes (85 % decrease);

0,5 mV after 90 minutes (95 % decrease).

With 0.1 mg/kg of the known substance II, an initial ST segment elevation of 6.5 mV caused by occlusion alone was increased to

9.0 mV after 20 minutes (38 % increase)

which value remained unchanged after 40 minutes.

With 1.0 mg/kg of the known substance II, an initial ST segment elevation of 6.5 mV caused by occlusion alone was increased to

8.5 mV after 20 minutes (31 % increase);

8.0 mV after 40 minutes (23 % increase).

THAT it follows from these data that, when applied in a dose of 0.125 mg/kg, the present compound I effected a 95 % protection against hypoxy as indicated by a decrease of ST segment elevation, whereas the known compound II, when applied in doses of 0.1 and 1.0 mg/kg, respectively, under the same conditions, did not show any protection against hypoxy as indicated by a decrease of ST segment elevation in the electrocardiogram.

THAT the undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Done, this 20th day of June, 1980
at Darmstadt / Germany.

Karl-Heinz Becker